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WOUND CLOSURE AND OUTCOME IN EXTENSIVELY BURNED PATIENTS TREATED WITH CULTURED AUTOLOGOUS KERATINOCYTES

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Cultured autologous keratinocytes (CAK) have been heralded as a means to achieve more rapid closure of massive burn wounds. Despite the claimed benefits of this technology, we have failed to identify its positive impact on wound closure in extensively burned patients. Sixteen patients with a mean age of 29.7 years (range, 10–56 years) and a mean total body surface area burn of 68.2% (range, 42%–85%) underwent 22 applications of CAK supplied by a private laboratory. The keratinocyte grafts were applied to a mean of 15.9% of the body surface area (range, 4%–59%) at an average cost per patient of $43,705 (range, $9,800 to $161,000). The mean body surface area of definitive wound coverage by these grafts was 4.7% (range, 0%–18.6%). The mean length of hospitalization was 132 days (range, 50–275 days). The observed mortality was 12.5% (two patients). Our experience with this wound care approach has been assessed with respect to the extent of burn, the level of wound excision, and the site of CAK application.

IMPROVEMENTS IN resuscitation, ventilatory support, and nutritional management have contributed to increased patient survival following massive thermal injury. Ultimate patient outcome remains dependent upon early, timely closure of the burn wound, typically by the application of split-thickness autografts harvested from uninjured areas. Often patients with extensive thermal injury have a disparity between available donor sites and the areas requiring coverage. Additionally, because of the paucity of donor sites, multiple graft harvests from the uninjured areas may be necessary, yielding tissue of progressively inferior quality.

Several avenues have been pursued in an attempt to overcome the barriers to timely wound coverage in patients with massive thermal injury. Bilaminate artificial skin substitutes composed of synthetic dermal and epidermal analogues have been tried[1,2] but are susceptible to submembrane suppuration and provide only temporary closure since the application of thin split-thickness autografts is still required for definitive wound coverage. Attempts to prolong human allograft survival to achieve permanent coverage are fraught with the hazards associated with immunosuppression.

The availability of in vitro cultivated human keratinocytes has prompted many investigators to assess the usefulness of such tissue in wound management. Gallico and colleagues reported the first clinical experience with cultured autologous keratinocytes (CAK) in 1981,[3] and several other clinical reports have followed.[4-6] This report describes our experience using this technology in patients with massive thermal injury.

MATERIALS AND METHODS

The U.S. Army Institute of Surgical Research is a 40-bed burn intensive care unit that admits more than 210 patients per year. The average burn size is approximately 30% of the total body surface area, and 35% of our admissions suffer concomitant inhalation injury.

Patients admitted with burns in excess of 40% total body surface area were eligible for entry into the protocol, approved by the Institutional Review Board of the U.S. Army Institute of Surgical Research and the U.S. Army Surgeon General's Human Use Review Board. After obtaining informed consent, and within 48 hours of admission, a 2 cm² full-thickness biopsy was obtained from an uninjured body site and sent to the laboratory of Biosurface Technology, Inc., in Cambridge, Massachusetts. Cell culturing techniques were essentially those described by Rheinwald and Green and are beyond the scope of this report.[7] Typically, 3 to 4 weeks of preparation were required before the delivery of cultured epithelial autografts mounted on petroleum jelly gauze backings.

Sixteen patients were entered into the protocol, and the majority were taken to the operating room for excision and split-thickness autografting from available donor sites during

the cell culture period. Once available, CAK grafts were applied to freshly excised wound beds; however, several patients had undergone prior excision and application of cadaver allograft at the site of CAK application. The keratinocyte grafts, measuring approximately 25 cm², were carefully applied to a non-bleeding, viable wound bed, stapled into place, and further secured with bridal veil netting and dry laparotomy pads. These dressings were moistened twice daily by topical application of an antibiotic solution containing vancomycin, polymyxin, bacitracin, and neomycin in concentrations previously shown not to be toxic to the CAK. The wounds were inspected for evidence of infection or excessive serous drainage on postoperative day 3. Petroleum jelly gauze backings were removed between postoperative days 7 and 10, and thereafter protective dressings were applied to the wound surfaces.

Active range of motion and ambulation were delayed for approximately 3 weeks following the CAK placement because of the fragility of the applied sheets of cells. Measurement and application of compression garments were withheld during this time period as well.

A photographic record was maintained of the operative wound bed, CAK application sites, wound bed appearance at the time of dressing removal, and the appearance at the time of removal of the gauze backings (approximately postoperative day 10) and at discharge.

Graft take, or extent of successful engraftment, was estimated by two observers viewing the wounds independently. The estimated percentage of body surface area definitively covered was determined by the product of the percentage take at discharge and the total surface area of cultured epithelial autografts applied as related to the total patient body surface area.

RESULTS

Sixteen patients with burn sizes exceeding 40% of the total body surface area (TBSA) underwent 22 applications of cultured keratinocytes during the period January 1990 through April 1991 (Tables 1 & 2). Thirteen patients underwent a single application of cultured keratinocytes, two patients underwent two applications, and one patient underwent four procedures. The mean age of the treated patients was 29.7 years (range, 10–56 years) with a mean burn size of 68.2% TBSA (range, 42%–85%). Eleven patients had documented inhalation injury requiring mechanical ventilatory support. The mean length of hospitalization was 132 days (range, 50–275 days). Two patients died, yielding a 12.5% mortality.

Keratinocyte grafts were applied to a mean of 15.9% of the body surface area (range, 4%–59%) at a mean cost of $43,705 (range, $9,800–$161,000) (Table 3). The mean percentage of initial keratinocyte engraftment was 64.4% (at time of gauze backing removal). The mean percentage of keratinocyte engraftment at the time of discharge was 46.7%, suggesting an element of late graft loss. Because the ultimate goal of this technology is definitive body surface area wound coverage, and because small surface areas of keratinocyte application with excellent engraftment may tend to skew the data in an artificially favorable direction if only the mean percentage of engraftment is considered, it was felt that an assessment of actual body surface area wound coverage was a more objective means of assessing the impact of cultured keratinocytes. In this cohort of patients, the actual body surface area of definitive wound coverage was 4.7% of the total body surface area rather than 7.5%, which would erroneously be calculated by considering mean surface area of application (15.9%) and mean percentage engraftment (46.7%).

To further delineate our experience with this wound care approach, the success of engraftment was assessed with respect to the level of wound excision and the extent of burn. Table 4 summarizes our experience with respect to the excisional wound bed. Mean patient age and burn size were similar between both groups and each had evidence of delayed graft loss. Mean initial engraftment was 52.6% for the fascial excision group and 73.5% for the deep dermal/fat excision group, whereas final take

Table 1
Summary of cases

Patient No.	Age (years)	TBSA Burn (%)	Inh. Injury	Patient BSA (m²)	Excision Bed	Percent BSA App.	Percent Engraftment Initial	Percent Engraftment Final	BSA Cover	Cost ($)	Outcome
1	26	44.0	Positive	1.69	Fascia	4.0	60.0	69.3	2.8	9,800	Lived
2	23	42.0	Negative	1.70	Dermis	6.0	80.0	82.5	5.0	14,350	Lived
3	46	61.0	Positive	1.74	Dermis	8.7	90.0	50.0	4.4	21,350	Lived
4	18	64.0	Negative	1.90	Dermis	5.4	87.0	85.0	4.6	14,350	Lived
5	25	58.5	Negative	1.79	Dermis	6.6	70.0	65.7	4.3	16,450	Lived
6	48	51.5	Positive	1.64	Dermis	8.8	85.0	88.6	7.8	20,300	Lived
7	26	82.0	Positive	2.09	Dermis	9.9	50.0	0.0	0.0	29,050	Lived
8	29	77.5	Positive	1.94	Dermis	13.9	80.0	71.5	9.9	37,800	Lived
9	19	77.0	Positive	1.87	Dermis	24.3	85.0	0.0	0.0	63,700	Lived
10	23	76.0	Positive	1.94	Dermis	24.5	75.0	76.0	18.6	66,500	Lived
11	56	85.0	Positive	2.55	Fascia	31.5	10.0	0.0	0.0	112,350	Died
12	35	70.0	Negative	2.09	Fascia	11.2	43.0	12.3	1.4	32,900	Died
13	18	75.0	Positive	1.94	Fascia	59.2	30.0	1.7	1.0	161,000	Lived
14	35	80.0	Positive	1.88	Fascia	21.1	80.0	14.8	3.1	55,650	Lived
15	39	75.0	Negative	1.87	Fascia	12.0	60.0	44.4	5.3	31,500	Lived
16	10	73.5	Positive	1.18	Fascia	7.4	85.0	85.0	6.3	12,250	Lived

Table 2
Summary of USAISR Experience

16 Patients—22 applications	
Mean age (years)	29.7 ± 3.1 (range, 10–56)
Mean TBSA burn (%)	68.2 ± 3.3 (range, 42%–85%)
Mortality (%)	12.5 (2 patients)

Table 3
Total patient population

Mean TBSA application (%)	15.9 ± 3.5 (range, 4–59)
Mean percentage engraftment (%)	46.7 ± 8.9 (range, 0–88)
Mean TBSA definitive wound coverage (%)	4.7 ± 1.2 (range, 0–18.6)
Mean cost per patient	$43,705 ± $10,348 ($9,800–$161,000)

was quantitated at 32.5% and 57.5%, respectively. In addition, despite application of CAK to a larger proportion of the body surface area (20.9% vs. 12%) patients undergoing fascial excisions had a smaller area of definitive wound coverage (2.8% vs. 6.1%) and at a greater cost ($59,350 vs. $31,450) than patients undergoing deep dermal/fat wound excisions; the difference approaches statistical significance. Table 5 summarizes the data with respect to the extent of burn injury. Again, the ages of the two patient groups were similar; however, patients with burn sizes in excess of 70% of the body surface area had a lesser extent of initial engraftment (59.8% vs. 72.0%) compared with patients with smaller burns. Furthermore, late graft loss in patients with large burn sizes contributes to the significant difference in final engraftment (30.6% vs. 73.0%) between the two groups. The net result was a similar extent of definitive wound coverage (4.6% vs. 4.8%) despite more than threefold greater area of application of keratinocytes (21.5% vs. 6.6%, $p < 0.05$), at significantly greater cost ($60,270 vs. $16,100, $p < 0.05$) in the patients with burns exceeding 70% of the body surface area.

DISCUSSION

An increasing number of extensively burned patients are being successfully resuscitated and supported during the early postinjury period. Although modern intensive care management has contributed substantially to improved survival, ultimate outcome is still dependent upon definitive closure of the burn wound. The frequently encountered disparity between available donor sites and burn areas requiring coverage has stimulated interest in alternative means of wound coverage.

Rheinwald and Green's report of the successful cultivation of human keratinocytes in vitro encouraged many investigators to evaluate the use of such tissues for burn wound closure.[7] The use of cultured keratinocytes by Gallico and O'Connor in the treatment of two children with burns of more than 95% TBSA was the first report of successful application of the technique.[3] One half of the burned area of each patient was covered with CAK, and the authors reported a 60% to 80% successful engraftment when applied to healthy beds of granulation tissue. The report encouraged the use of cultured epithelial autografts for burn wound management, and several subsequent reports have touted the attributes of this technology.

DeLuca and colleagues reported a multicenter experience of burn wound treatment using cultured autologous epithelium.[4] Their report emphasized the correlation between patient age and the time required to activate keratinocytes, which was attributed to the lower colony forming efficiency of keratinocytes isolated from older donors. Also, a disparity in successful engraftment related to patient age was noted; patients younger than 18 years of age had a 47 % average take, whereas those older than 18 years of age had a 28% engraftment. This was partially attributed to the decline in growth potential seen with aging.

Teepe and associates reported the experience of 17 patients treated with cultured keratinocytes, and compared the patients with respect to wound bed preparation.[5] Early wound excision and preparation with cadaver allograft as a biologic dressing resulted in an average of 47% engraftment success compared with grafts applied to nonexcised, chronic granulating wounds which resulted in a 15% average take. The difference was attributed to extensive bacterial contamination of the chronic granulating wounds. Even though such contamination

Table 4
Data analysis with respect to excisional wound bed

	Fascial Excision (N = 7)		Dermal Excision (N = 9)	
Mean age (years)	31.2 ± 5.7	(1–48)*	28.5 ± 3.7	(10–56)
Mean burn size (%)	71.8 ± 5.0	(44–85)	65.5 ± 4.5	(42–82)
BSA application (%)	20.9 ± 7.3	(4–59)	12.0 ± 2.8	(5.4–24.5)
Mean percentage of engraftment				
Initial	52.6 ± 9.4	(10–85)	73.5 ± 4.5	(50–85)
Final	32.5 ± 12.9	(0–85)	57.7 ± 11.6	(0–88)
BSA definitive coverage (%)	2.8 ± 0.9	(0–6.3)	6.1 ± 1.9	(0–18.6)
Mean patient cost	$59,350 ± $21,438		$31,540 ± $6,821	
	($9,800–$161,000)		($14,350–$66,500)	

* Ranges given in parentheses.

Table 5
Data analysis with respect to patient burn size

	<70% TBSA (N = 6)	>70% TBSA (N = 10)
Mean age (years)	31.0 ± 5.2 (18–48)	29.0 ± 4.1 (10–56)
Mean burn size (%)	53.5 ± 3.7 (42–64)	77.1 ± 1.4 (70–85)*
Mean BSA application (%)	6.6 ± 0.8 (4.0–8.8)	21.5 ± 4.9 (7.4–59.2)†
Mean percentage of engraftment		
Initial	72.0 ± 5.5 (10–85)	59.8 ± 7.8 (50–87)
Final	73.5 ± 6.0 (50–88)	30.6 ± 11.1 (0–85)‡
Mean BSA definitive coverage (%)	4.8 ± 0.7 (2.8–7.8)	4.6 ± 1.9 (0–18.6)
Mean patient cost	$16,100 ± $1,742 ($9,800–$21,350)	$60,270 ± $14,256† ($12,250–$161,000)

* $p < 0.001$; † $p < 0.05$; ‡ $p < 0.01$; ranges given in parentheses.

was not quantified, it appears to be a plausible reason for the observed results.

Most recently, Munster and colleagues reported their experience with seven patients with a mean burn size of 69.6% of the total body surface area.[6] An average of 18% of the body surface area was covered with cultured keratinocytes, and the reported success of engraftment was 75%. Furthermore, the authors, comparing a series of historic controls, demonstrated no difference in the length of hospital stay, total number of surgical procedures, or cost.
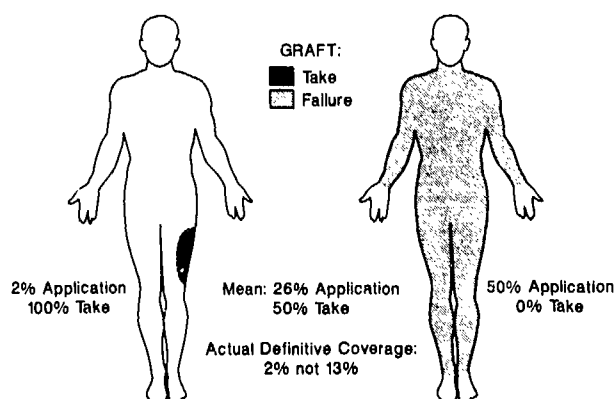
On first inspection, our results with cultured keratinocytes, using a protocol of fresh excision and immediate application of the grafts, are quite similar to the previous reports. Overall graft take in this cohort of patients was a mean of 47%. However, we believe a more objective means of assessing the impact of this technology is to determine the actual body surface area of wound closure achieved by CAK application. Figure 1 illustrates the confusion that can be generated by expressing take as a product of the cohort average percentage of engraftment times the cohort average extent of body surface area grafted, as opposed to actual body surface area of wound coverage as we have reported. In this cohort of burn patients, only 4.7% (range, 0%–18.5%) TBSA was definitively covered with CAK despite application to an av-

Figure 1. Assessment of impact of cultured autologous keratinocytes on wound closure is best reported as body surface area of definitive coverage rather than mean engraftment versus mean surface area of application.

erage of 15.9% of the body surface. Further examination of the data reveals marked late graft loss (59.8% initial, 30.6% final engraftment) yielding small areas of definitive wound closure (4.6%), at such high cost ($60,270, in patients with extensive thermal injury (>70%). One must ask whether this small proportion of skin coverage at such high cost (more than $13,000 for each 1% covered) can be claimed to contribute, in a significantly positive manner, to the outcome for extensively burned patients.

DeLuca's data,[4] when analyzed with respect to burn size, reveals that patients with burn sizes greater than 70% TBSA had an average take of 9.2% per application, whereas patients with burn sizes less than 70% had a 23.1% average take per application. In our cohort of patients, the tendency for patients with large surface area burns having less successful engraftment of CAK was also observed. This demonstrates the need for multiple applications at substantial cost to achieve definitive wound coverage for patients with more extensive burns.

Loss of applied split-thickness skin grafts typically occurs as a consequence of the individual or combined effects of mechanical shearing, technical misadventures, gross nutritional imbalances, or infection. In this cohort of patients, efforts were made to minimize the risks of these factors. All surgical excisions and CAK applications were supervised intraoperatively by a senior staff surgeon. In all but a few patients, posterior body surface area applications were avoided to minimize shear effects. Aggressive nutritional support with frequent reassessment of the adequacy of that support was provided to all patients. Furthermore, topical antibiotic solutions, nontoxic to CAK, were applied to the graft sites to lessen the extent of bacterial colonization and thereby reduce the risk of infection.

Patients suffering loss of applied cultured keratinocytes appeared to fall into two groups: those with early keratinocyte loss marked by significant separation or disappearance of the grafts at the time of initial gauze backing removal, or later gradual erosion of the applied grafts (Fig. 2). Patient 11 illustrates the problem of early graft loss. The patient, a 56-year-old man with an 85% total body surface area burn, was taken to the operating room for fascial excision and keratinocyte application to

Figure 2. Photograph of patient 8 with evidence of late graft loss. Arrow depicts area of erosion that progressed in size, ultimately necessitating application of split-thickness skin grafts to achieve wound closure.

Figure 3. Photograph of patient 13 with evidence of early graft loss. Arrows depict complete separation of previously placed CAK grafts. Similar "rejection" of additional grafts progressed over the ensuing weeks, with ultimate complete loss of applied grafts.

his anterior chest and abdomen on postburn day 28. At the time of removal of the gauze backings on postoperative day 9, only 10% of the applied cells were noted to be adherent. Further total graft loss was observed at the time of his death on postburn day 41. Patient 9 illustrates the problem of delayed loss of keratinocytes previously perceived to be adherent. This patient, a 19-year-old man with a 77% total body surface area burn, underwent excision of his left arm and both legs at the level of deep dermis and fat on postburn day 28. Initial graft take was assessed at 85%; however, over the course of the next several weeks, gradual graft erosion occurred and progressed to complete loss of all applied CAK.

Multiple factors are likely responsible for keratinocyte graft loss. It is possible that late graft loss occurs because of the lack of durability. This fragility may be attributable to a lack of dermis, particularly in patients in whom excision generates a fascial wound bed, and a paucity of anchoring fibrils as described in the study by Compton and colleagues of the morphology of cultured epithelial autografts applied to pediatric patients whose burn wounds were excised at the fascial level.[8] This report noted that 3 to 4 weeks following keratinocyte application a completed dermal/epidermal junction was present, as were anchoring fibrils; however, more than a year was required before full maturation and development of anchoring fibrils in sufficient density reduced the susceptibility to epidermal separation. Furthermore, during the first year, the underlying wound beds undergo remodeling such that by 2 to 3 years following graft application the subjacent connective tissue resembled dermis, which also contributed to greater durability. Unfortunately, to our knowledge no such data are available in the adult patient population.

Heavy microbial density, as described by Teepe and colleagues,[5] may well contribute to both early and late keratinocyte graft loss. Although none of our patients had demonstrable wound bed infection as delineated by histologic evidence of invasion of bacteria into viable tissue, all patients were colonized with *Staphylococcus aureus* and, in some cases, *Pseudomonas aeruginosa*, despite the regular use of topical antimicrobial solutions. There did not appear to be a correlation between success and failure of keratinocyte engraftment and microbial colonization; however, it should be noted that neither histologic examination nor quantitative cultures were obtained in this cohort of patients. Attempts to delineate the impact of micro-organisms on the success of keratinocyte engraftment will require more controlled quantitative assessments of bacterial density. Furthermore, many authors recommend the early excision and immediate application of cadaver allograft on wound sites that are to be covered with cultured keratinocytes, so as to optimally prepare the wound bed.[14] It is our impression that excision to a viable wound bed and immediate application of cultured keratinocytes will serve to minimize any adverse impact of microbial colonization on engraftment.

Finally, it is interesting that six of the ten patients with burn sizes exceeding 70% of the total body surface area sustained either early or late significant graft loss in the absence of demonstrable wound bed infection. The appearance, in several patients (Fig. 3), suggested that some types of immune phenomena might be responsible for graft loss. Whether this is related to the persistence of fetal bovine antigens from the culturing phase as suggested by Meyer and colleagues,[10,11] or a circulating antiepidermal antibody that increases as burn size increases,[12] remains to be investigated.

Although our overall patient experience with this technology has been less than encouraging, particularly in patients with large burn sizes (>70% of the total body surface area) and in those in whom excision produced a fascial wound bed, the data do reveal striking variability of success with keratinocyte engraftment. In several patients, this wound care approach was highly effective in achieving wound closure. Consequently, future investigation should focus upon specific causes of graft failure

and the development of techniques by which engraftment success can be optimized. Additionally, the potential merging of this technology with the in vitro cultivation of viable dermal analog, as described by Hansbrough and colleagues, may result in the production of a bilaminate skin product that would have greater durability, increase engraftment success, and achieve definite wound closure.[13,14] Timely definitive closure of the burn wound remains a principal determinant of survival. Techniques that overcome a donor site-burn wound disparity will significantly contribute to the prompt closure of extensive burn wounds. To date, on the basis of this experience, we conclude that the use of cultured autologous keratinocytes has not significantly reduced the time required to achieve closure of massive burn wounds and consequently has exerted no demonstrable effect on the outcome of extensively burned patients.

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DISCUSSION

Dr. Linda G. Phillips (Galveston, Texas): I would like to begin by commending the authors on a well executed and important contribution to our understanding of the role of cultured autologous keratinocytes in the treatment of massively burned patients. The authors make several very important statements regarding the use of the cultured autologous keratinocytes. They note that the grafts took best on a tangential or full-thickness skin excision compared with fascial excision. I wonder if Doctor Rue can comment on whether this difference is due to superiority of the bed itself or to a less traumatized patient not requiring a fascial excision?

The authors state that the grafts were not placed on dorsal surfaces to minimize shear. However, I wonder if any of these grafts were placed near flexion creases, the flanks, or the medial limbs, which would also allow for shear?

The authors note that the grafts were friable, and so early motion was delayed. Was any decreased range of motion noted? This would be a contraindication for the use of the cultured autologous keratinocytes.

With the lack of grafted dermis and the prolonged inflammatory phase caused by the unstable scar, long-term joint contractures could be expected. Was any increase noted in this contracture rate?

Finally, in our institution, noting the instability of these grafts, we assayed blister fluid where shearing occurred. The fluid had the highest concentration of thromboxane of any blisters we examined, including those from frostbite and burns. I would like to suggest to the authors that late ischemia caused by arachidonic acid metabolites may at least contribute to late graft loss.

Dr. Anthony A. Meyer (Chapel Hill, North Carolina): I would like to compliment the authors on the work done. Taking care of these patients with this cultured skin is very difficult, and they did a very nice analysis of their results.

I think the questions that I have are in terms of the tangential excision or excision to fat. Was there anything used to try to guarantee that what you saw healing on the wound was not skin grown from residual dermal structures?

And then, finally, are you considering growing your own skin as an alternative to defray the incredible cost of purchasing the cultured skin from commercial sources?

Dr. Ronald G. Tompkins (Boston, Massachusetts): I have a comment and two questions. Our experience at the Shriners Burns Institute in Boston is very similar to this report in regard to overall eventual take and cost. If the cells were not at such an extraordinary cost, I am sure we would not have had this presentation and discussion. If the cells cost one hundredth as much, they would be a reasonable alternative worth developing further.

At least in our view, these cells are indicated only in patients with as small as 5% or 10% of the body surface area available as donor site. Possibly in adults, the cells could be useful in injuries exceeding 70% TBSA but we have had essentially no experience with the cells at the MGH in the adult unit.

Our frustration is that the contribution to overall wound closure is frequently not clinically very useful. Likewise, the likelihood of successful take tends to decrease with the burn size which massively burned patients are exactly the patient for whom these cells are needed.

I have two questions, one statistical in nature. Have you analyzed the likelihood of take versus burn size in a logistical regression fashion? It is really those patients with 85% to nearly 100% burn injury for whom these cells are really useful at all.

Second, do you have any biopsy information that would address the controversy regarding the dermal-epidermal structure development and allow us to understand the delayed graft loss somewhat better?

Dr. Jeffrey R. Saffle (Salt Lake City, Utah): I enjoyed the presentation and the refreshing candor which the folks from Brook brought to this topic.

In our experience, it is clear that cultured autologous keratinocytes do nothing to clear up wound infections or sepsis, and in fact really cannot be used in that setting. And we have had the frustrating experience several times of sending the skin off to be cultured, only to have the patient get into trouble before the skin is available, so that we were never able to use it.

In your patients presented here, all of the cases were covered with autologous kin, and I wonder if there were other patients for whom you sent the skin off only never to have an opportunity to use it, and whether the costs of that process were figured into the figures that you reported here.

Dr. Loring W. Rue, III (Closing): I would like to thank all the discussants for their comments. First, I would like to address Doctor Phillips' questions. With regard to the issue of dermal versus fascial excision and whether or not we can attribute some of these results from persistent residual epithelial elements, I think the group at Cornell previously reported on the use of cultured allogenic keratinocytes placed on various wound beds. It was initially interpreted that there was good take and resurfacing of these wounds. Subsequently, when the areas were biopsied and DNA fingerprints performed, it was found that basically the patient's own skin was present and this probably resulted from regeneration of residual epithelial appendages. So probably there may be an element of that involved with the results we present here today.

The shear problem is a significant one. Many of these patients did have the grafts placed across flexion creases, and certainly this might hinder the take of those applications. There was some decreased range of motion noted, particularly related to the fact that the patients were immobilized for a long period of time so as to optimize take of the cells.

From your studies in Galveston, the increased levels of thromboxane A_2 and prostaglandin$_E$, I believe does raise the question whether or not there is an ongoing inflammatory process that might also hinder the optimal take of these grafts.

Doctor Meyer, in response to your question about whether or not we confirmed the presence of the cells by biopsy, we did not do that. However, we have considered an amended protocol to actually incorporate biopsy of graft sites into the study.

In response to Doctor Tompkins' comments, we would like to do some immunofluorescent studies to specifically examine the amounts of type 4 and type 7 collagen present in these patients, which may have implications with respect to graft loss.

As for Doctor Meyer's question of whether or not we had considered growing our own keratinocytes, we have indeed evaluated that option; however, it should be pointed out that there are a number of indirect costs and costs unrelated simply to the preparation of the product. For example, the costs of various employees and their benefits, so we have not really given that serious consideration at this point.

Doctor Saffle, in response to your question, we did have three patients whose biopsies were forwarded in hopes of using cultured keratinocytes to resurface their wounds. Unfortunately, these patients died before graft preparation so we had three cases that meet the criteria you inquire about.

I want to thank the discussants for their comments and I want to thank the Association for the opportunity to present our data today.